Antibiosis and Fungistasis of Soil Microorganisms

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A logical approach to the successful control of root diseases requires as deep an understanding as possible of the complex ecosystems existing in the soil horizons occupied by roots, where a large variety of organisms interact in such a way that a relatively stable community develops, provided there is no major change in the environment. In fact it is usually found that changes in environmental factors are constantly taking place, creating a dynamic system. Particularly well marked are seasonal changes reflected directly by changes in temperature and moisture status of the soil, and indirectly by plant growth and nutrient supply. In soil, probably more than in any other habitat, growth and development of individual species is dependent upon others, not only for supplies of nutrients, but also because of the growth-regulatory activities of a wide range of metabolites that are excreted into the environment and have been aptly termed environmental hormones or ectotrines by Lucas (1947). These metabolites may have beneficial stimulatory or growth-regulatory activity, as nutrients, growth substances, or vitamins, or they may have inhibitory effects and then be described as antibiotics. Often the same substance acts in more than one role, the expression of its effects depending upon the species affected, concentration, and perhaps our interpretation of the situation. We can now define antibiosis as the condition in which one or more metabolites excreted by an organism have a harmful effect on one or more other organisms. It may be considered as a facet of the more general condition of antagonism. Antagonism embraces competition, antibiosis, predation, and parasitism. While these phenomena may be defined so as to avoid confusion, the comparative rarity in nature of one unaccompanied, to a greater or lesser degree, by at least one of the others, leads inevitably to difficulties as soon as we attempt to analyse a natural situation. These difficulties are further aggravated by the fact that in soil we can hardly ever afford the luxury of restricting our vision to two species.

TYPES OF ANTIBIOTIC INTERRELATIONS.—In the simplest model situation, species *a* produces a substance which is in some way detrimental to species *b*, and as a consequence the growth or reproduction (or both) of *b* is retarded or stopped. Since the species pairs will almost certainly be competing for at least some factors of the environment, usually nutrients, a secondary effect of antibiosis will be to reduce the competitive ability of species *b* relative to species *a*.

In our consideration of the biological control of root diseases, we will be thinking most often in terms of antibiosis between different microorganisms. But substances secreted by microorganisms are also known to have a directly harmful effect on plants without any invasion of their tissues. Reduction in root elongation, as may be caused in wheat and other plants, for example, by *Azotobacter chroococcum* (Krasilnikov, 1939), or the modifications in root growth and morphology resulting from ectotrophic mycorrhizal infection, are probably the direct effect of growth substances produced by the microorganisms and, unless demonstrably harmful, are excluded from this discussion. That some modification of root morphology may be a normal concomitant of growth in an environment inhabited by microorganisms has been shown by the experiments of Bowen and Rovira (1961); it need not be assumed that such changes are necessarily deleterious to plant growth. Numerous reports of damage to seedlings due to toxic metabolites of microorganisms have been made, particularly by Russian authors (Mirchink and Greshnykh, 1962; Stepanova and Fish, 1958), who have emphasized the effect of manurial practices on the activity of these organisms. A specific case of damage caused by toxin production was brought to light by Steinberg (1950) in his classic investigation of frenching of tobacco. It would seem probable that more or less severe damage to seedlings not infrequently results from the production of toxins by soil microorganisms. In many instances a precondition of this form of antibiosis may be selective stimulation of the toxin producers by plant root exudates. Such a stimulation was shown by Kerr (1956) to be related to the degree of damage produced. Kerr's experiments, using cellophane bags to prevent direct contact between seedlings and the fungi studied, *Pellicularia filamentosa* and *Sclerotinia homoeocarpa*, gave a clear demonstration of the ability of these fungi to cause severe stunting and necrosis to roots with which they were not in contact. It is worth remembering, also, that several purified antibiotics, including some of fungal origin, are known to have phytotoxic properties (Wright, 1951).

The reverse situation to that considered above, where the plant exercises an antibiotic effect on the microorganism, is probably of no less importance. Reference has been made by other contributors to the presence of specific inhibitory substances in root exudates, and their possible role in resistance to root infection. There is clear evidence that the seed itself may exude antibiotics which have a significant effect on the establishment of organisms within the rhizosphere. Of particular practical significance is the toxicity of legume seeds towards *Rhizobium* species (Bowen, 1961). Antagonism in the rhizosphere between *Rhizobium* and other organisms was originally suggested as the reason for obtaining unsatisfactory nodulation of subterranean clover in certain Australian soils (Hely, Bergerson, and Brockwell, 1957); but more recent work (Thompson, 1961) indicates that toxic seed exudates are at least partly responsible and that pelleting is effective in improving nodulation because it separates the *Rhizobium* to some extent from the seed coat.

The next order of complexity to that in which two organisms are involved in an antibiotic relation is that between three or more species, as for example, where we have an antibiotic producer *a*, a pathogenic organism *b* susceptible to the antibiotic, and a plant *c* acting as a host for the pathogen. Such a system is still relatively simple and does not involve the kind of interactions which have been postulated by Park (1960) and which must give a truer picture of the likely complexity of soil interrelations. The equilibria between soil organisms are so complex and finely balanced that we should not be too optimistic about our ability to analyse accurately any antibiotic system involving more than three interrelated members.

Certain varieties of crop plants resistant to root pathogens may owe their resistance to peculiarities in their root exudates, favouring the establishment of rhizosphere organisms antibiotic towards pathogenic fungi. Timonin's study (1940, 1941) of the resistance of the flax variety Bison to fusarium wilt showed that differences in the rhizosphere fungi of susceptible and resistant varieties were correlated with differences in susceptibility to hydrocyanic acid, which was secreted in appreciable quantities by roots of resistant varieties, but only in traces by susceptible plants. *Trichoderma viride* was shown to be actually stimulated by the presence of hydrocyanic acid. Subba-Rao and Bailey (1961) found an association between the high incidence of *T. viride* in the rhizoplane and resistance to verticillium wilt in two resistant varieties of tomatoes, but not in a third, from the rhizoplane of which *Trichoderma* was absent. Although no antibiosis was shown between their isolates of *Trichoderma* and *Verticillium* in vitro on a medium not containing root exudate, the fact that some protection was afforded to susceptible varieties inoculated with *Trichoderma* suggested that in some instances there may be a causal relation between predominance of this fungus in the rhizoplane and resistance.

Another example of the association of organisms inhibitory to a pathogen, with a resistant variety, has been provided by work on varieties of pigeon-pea resistant and susceptible to wilt caused by *Fusarium udum* (Agnihothrudu, 1955). In seven different soils from 13 to 33% of rhizosphere isolates from the resistant variety strongly inhibited *F. udum*, whereas organisms isolated from the rhizosphere of the susceptible variety were inhibitory from one soil only, and these constituted only 6% of the isolates. The active organisms were all species of *Streptomyces*, and it is of interest that they were much more effective in vitro in media containing root extracts from the resistant variety than with extracts from the susceptible variety.

The probable importance of root exudates in determining antibiotic relations is also shown by Buxton's (1960) studies with pea wilt. It is perhaps worth emphasizing here the dangers of assuming that high counts of organisms capable of producing antibiotics in vitro gives proof of significant antibiotic activity in the rhizosphere. Such counts only indicate the potentialities of the population and may bear no causal relation to the occurrence of a pathogen.

THE OCCURRENCE AND SIGNIFICANCE OF ANTIBIOTICS IN SOIL.—In my discussion of antibiotic relations in soil, I have been assuming that antibiotics are produced in soil and that they have a significant and even determining influence on the ecosystems therein. This assumption is not one which has received universal acceptance, so it is necessary to examine some of the evidence upon which it is based. I think it is important to remind ourselves that natural soils, far from being homogeneous, are composed of a complex of discontinuous microhabitats. These microhabitats may differ widely one from another in any of the factors which determine microbial behaviour within them. Experiments designed to determine the fate of antibiotics added to rather large masses of soil or to evaluate the antibiotic-producing potentialities of specific organisms in variously treated soil masses have an undoubted value, but are largely irrelevant to the conditions within microhabitats which determine the success of individual organisms.

It has been unequivocally demonstrated by Wright (1956*a*,*b*) and others that antibiotic production will take place in organic substrates, such as pieces of straw or seedcoats, buried in soil. It cannot be doubted in these instances, where the quantities of antibiotic involved are large enough for extraction and characterization, that concentrations sufficient to influence the pattern of microbial colonization of these substrates must occur. Similar concentrations are also likely to occur in smaller pieces of organic matter in which the quantities of antibiotics produced are too small for detection by conventional means. Perhaps the development of microtechniques, such as used by Stevenson (1956) and Rangaswami and Ethiraj (1962), will enable us to extend our knowledge of antibiosis in microhabitats.

The focal points of our interest in root diseases are the rhizosphere and rhizoplane, where antibiosis, if it occurs, may be expected to be crucial in the establishment or failure of infection. Direct evidence for the presence of specific antibiotics in the rhizosphere has

not been easily forthcoming (Kalyanasundaram, 1958). This is hardly surprising considering the difficulties attendant even upon the detection of individual constituents of root exudates in soil, which must be produced in quantities large relative to those of any one antibiotic. Indirect evidence is, however, accumulating that antibiotic production in the rhizosphere may have a determining influence on root infection. Reference has already been made to instances where the occurrence of antibiotically active organisms in the rhizosphere may explain the resistance of certain varieties of crop plants to root disease. The differences in the occurrence of a disease in contrasting types of soil may also be explained by antibiosis. Rishbeth's work (1950, 1951*a*,*b*) on a root disease of pines caused by *Fomes annosus*, provides a well-documented example. The spread of this disease was much more rapid in alkaline soils in East Anglia than on more acid heath and woodland soils. Superficial growth of *Fomes annosus* was abundant on roots in the alkaline soils but absent or feeble in the more acid soils. Roots in the latter soils were found to be colonized by *Trichoderma viride*, which was demonstrated to have a marked in-vitro antibiotic effect on *Fomes annosus*. A substantially similar situation has been found by Moreau and Schaeffer (1959) in the Jura region of France. The difficulties of interpretation of observations and experimental results are well illustrated by the examples given involving *T. viride*. This fungus, in addition to producing antibiotics, is known to have a high growth rate and competitive ability and also to possess potentialities for parasitism upon other fungi.

Widespread Soil Fungistasis.—Consideration of the possibility that antibiotics may accumulate in soil sufficiently to give the soil as a whole antibiotic properties, brings us to the difficult question of a widespread soil fungistasis. It is to the work of Dobbs and Hinson (1953), just 10 years ago, that the general recognition by mycologists of the problem of soil fungistasis is due, although isolated observations, which may now be explained by the phenomenon, had been made earlier. They found complete inhibition of germination of spores of *Penicillium frequentans*, buried in soil in folds of cellophane. This inhibition, which occurred in all 13 soils tested in the original work, could be overcome by the addition of glucose to the soil. Their original findings have now been confirmed and extended (Hinson, 1954; Dobbs, Hinson, and Bywater, 1960; Dobbs and Griffiths, 1961, 1962; Jackson, 1958*a*,*b*). The results make it certain that the spores of most fungi do not readily germinate in natural soil unless stimulatory substances, usually associated with plant residues or living plant roots, are present in sufficient concentration. Failure to germinate in soil is not usually due directly to a deficiency of nutrients.

Lingappa and Lockwood (1961) have criticised the interpretation of some of the experimental work on soil fungistasis in which cellulose film or agar has been used on the grounds that these substances may provide a substrate for the growth of antagonistic organisms. Their criticism cannot detract from the validity of the many related observations which confirm the existence of soil fungistasis. Their discovery that bacteria and actinomycetes growing from a mixed soil population on an agar surface for a short period could influence fungal spore germination at a distance of at least 2 mm in the absence of high concentrations of nutrients, must strengthen the case for accepting the significance of antibiosis in soil. The same workers showed that rather high concentrations of alcohol extracts of fresh spores of *Ustilago zeae* had an inhibitory effect on the soil microflora, but strongly stimulated growth at lower concentrations. Lingappa and Lockwood (1962*b*) later reported the observation of areas of stimulation of bacteria in the vicinity of fungal spores added to soil. These observations led them to the hypothesis that individual spores might release nutrients into the soil, stimulating the growth of antagonistic organisms in their vicinity, which then inhibit spore germination. This hypothesis might be valid for a short period after the spore first makes contact with the soil, but nutrient leak could not be expected to be sustained at a rate sufficient to maintain an active episporic flora for more than a few hours or perhaps days. Bacteria and actinomycetes, which can often be observed on the surface of growing hyphae, might easily affect hyphal growth by producing antibiotics.

Expression of Soil Fungistasis.—The most easily recognisable expression of fungistasis is inhibition of spore germination. Once germination has occurred, the germ tube is less susceptible to soil fungistasis, but subsequent development may be strongly influenced. In particular it has been observed that in unsterile soil, macroconidia of *Fusarium* spp. may germinate, but that after a short period of growth the germ tube is terminated by a chlamydospore (Jackson, 1960; Nash, Christou, and Snyder, 1961; Toussoun and Snyder, 1961). Sometimes the conidia themselves are converted into chlamydospores. In sterile soil, or in the absence of soil, chlamydospore formation is usually considerably delayed. Venkat Ram (1952) discovered that the production of chlamydospores in cultures of *F. solani* was much enhanced when the medium was inoculated with a soil isolate of *Bacillus licheniformis* or another, unidentified, bacterial isolate. Development of chlamydospores, as well as other morphological changes, in cultures of *F. oxysporum* have been attributed by Park (1961) to the accumulation of unidentified staling substances. It seems highly probable that many phases of fungal development in soil, particularly germination, vegetative growth, and onset and intensity of sporulation (Boosalis, 1962), are subject to some degree of influence by a variety of microbial metabolites acting as environmental hormones.

Death and lysis of hyphae, and less readily of spores, often follows contact with unsterile soil. What relation, if any, such lysis bears to soil fungistasis is not clear. It is known that a number of substances induce lysis (Carter and Lockwood, 1957) and that lysis may be common in cultures (Park, 1961). Reinoculation of autoclaved soil with isolates of *Streptomyces* spp.

known to be lytic in culture also induces lytic and fungistatic properties in the soil (Lockwood, 1958).

THE SOURCE OF SOIL FUNGISTASIS.—There is, as yet, no clear evidence of the origin or nature of soil fungistasis. When Brian (1960) discussed the possible role of antibiotics in soil, he concluded that although antibiotic accumulation was unlikely to occur in many of the soils in which fungistasis had been detected, antibiotic production nevertheless seemed the most likely explanation of fungistasis. He also pointed out that no detailed studies had yet been made of production in soil of the polypeptide antibiotics of bacteria, or of the antifungal polyenic antibiotics of actinomycetes, the chemical properties of which were in many ways rather distinct from those of the antibiotics which had so far received most attention.

It is true that the accumulation and activity of antibiotics in soil away from microhabitats of active metabolism has not generally been regarded as plausible, because of adsorption by clays and rapid degradation by the soil flora. But in recent studies of the adsorption of antibiotics in soil (Pinck, Holton, and Allison, 1961; Pinck, Soulides and Allison, 1961; Soulides, Pinck, and Allison, 1961, 1962) it has been shown that, for example, adsorption of streptomycin by the kaolinitic fraction of an unsterilized clay loam soil could result in the persistence of the antibiotic, in the adsorbed condition, up to 28 days after its introduction into the soil. A slow release during this period was apparent from the reaction of the bacterial flora. It would thus seem possible that an antibiotic adsorbed by the clay colloids might subsequently be released, perhaps after a pH change produced by local microbiological activity. While in the adsorbed state, an antibiotic would be protected from microbial degradation, but might still undergo physical or chemical breakdown (Soulides, Pinck, and Allison, 1962). Similar protection from the action of microorganisms through adsorption or inaccesibility has also been shown for humus and other organic molecules in soil (Ensminger and Gieseking, 1942; Rovira and Graecen, 1957; Esterman, Peterson, and McClaren, 1959).

Release of aromatic compounds resulting from the degradation of lignin by macrofungi (Henderson, 1960) may give rise to fungitoxicity, similar in some respects to soil fungistasis (Lingappa and Lockwood, 1962*a*). The action of these compounds seems to differ somewhat from normal fungistasis in that even at concentrations permitting germination, a strong inhibition of germ-tube growth was noted. Lignin monomers may be important in soils rich in organic matter, and their formation may also sometimes explain the action of soil amendments in disease control.

The similarities between fungal behaviour in staled cultures and in natural soil have suggested that soil fungistasis may be a form of general staling between both like and unlike organisms (Park, 1960). Recent support for this concept has been given by Griffin's (1962) experiments, which showed that autoclaved soil reinfected with several of a variety of microorganisms developed fungistatic properties, but that there was no correlation between ability to produce a fungistatic effect in soil and production of an antibiotic effect in culture. Griffin postulated that normal soil fungistasis may be partly a result of the general saprophytic activities of the soil microflora and partly that of toxic metabolites, other than specific antibiotic substances. It seems to me that description of the fungistatic situation in soil as a type of staling phenomenon is quite acceptable and not incompatible with the theories discussed. The situation will not, however, be materially advanced until we are able to isolate and identify with certainty at least some of the substances implicated. Whether we then choose to designate them antibiotics or staling substances is probably of no great importance and will depend only on precise definition of these terms. Perhaps Winter's (1961) conclusion, that widespread inhibiting conditions in soil must be ascribed to a complex of different inhibitory factors, including the synergistic effect of amounts which separately are below threshold levels of toxicity, may prove justified and help to explain some of the difficulties familiar to workers in this field.

THE SIGNIFICANCE OF SOIL FUNGISTASIS.—The ecological significance of the sensitivity of fungi to soil fungistasis, and the amelioration of inhibition by sufficient concentrations of nutrients would be difficult to overestimate. Sensitivity to a general and widespread soil fungistasis is of great survival value, particularly to the more ephemeral saprophytes and those fungi associated primarily with roots. Spores sensitive to fungistasis normally lie dormant in soil until stimulated by contact with soluble nutrients, when germination, followed by more or less extensive vegetative growth and often sporulation, can be achieved. The evolution of this mechanism can be regarded as an adaptation to a heterotrophic existence in a medium providing substrates which are discontinuous in space or time. This adaptation finds some parallel in the necessity for stimulation by a host, shown by parasitic angiosperms such as *Striga* spp. and by root-parasitic nematodes. These parasitic plants and nematodes, in common with some specialized fungal resting bodies, exhibit constitutive dormancy, in contrast to the fungal spores subjected to fungistasis, which may be regarded as exogenously induced dormancy.

ANTIBIOSIS AND FUNGISTASIS IN DISEASE CONTROL.—Most attempts to achieve disease control through antibiosis depend for their success on changing the equilibrium between the pathogen and its natural antagonists in favour of the antagonists. We have already seen how this may be the indirect outcome of the plant breeder's efforts, by selecting varieties whose rhizosphere offers a favourable environment to antagonists; and there is evidence that a difference in one gene may have a strong effect on the rhizosphere population (Elkan, 1962). Changes in the rhizosphere population can also be induced by foliar application of nutrients or other substances (Venkata Ram, 1960). Horst and Herr (1962) were able to detect a temporary increase in actinomycetes antagonistic to *Fusarium roseum* f.

cerealis in the rhizosphere of corn seedlings after the foliar application of urea.

The effects of adding organic amendments to the soil to change the microbial equilibrium so as to favour antagonistic organisms are well known and have been discussed by Dr. Garrett and others. Recent examples of the successful application of this method are the addition of chitin to the soil to control fusarium root diseases of radishes, beans, and peas (Mitchell and Alexander, 1961, 1962; Buxton, 1962). The control achieved in these cases appears to be associated with an increase in the numbers of bacteria and actinomycetes, which possess chitinolytic activity, and also inhibit or lyse fusaria. The value of chitin in selective media for the isolation of actinomycetes from soil was already known (Lingappa and Lockwood, 1960).

Attempts to introduce antagonistic organisms into an environment which is relatively permanent and stable and where they are not already present are unlikely to succeed. If, however, at least one factor of the environment is changed, the chances of successful introduction may be improved. Total sterilization of soil produces drastic changes, and the introduction of antagonists after such treatment may not be successful because of the unstable conditions created. More emphasis is now being placed on the use of less drastic methods such as treatment with low-temperature air and steam mixtures (Baker and Olsen, 1960; Baker, 1962) with the object of killing the more heat-sensitive pathogens but causing the least possible disturbance to the saprophytic flora. Inoculation of soil treated in this way with antibiotic-producing organisms may give useful results. The developing rhizosphere of a seedling plant does provide a new and changing environment which is normally colonized more or less fortuitously by seed surface and soil organisms. Suitable organisms may be introduced artificially into this environment. If their numbers are large relative to those of the normal colonizers, they may become established and sometimes remain in considerable numbers throughout the life of the plant (Brown, Burlingham, and Jackson, 1962). Such organisms are obviously well placed to influence the behaviour of root pathogens, and it is significant that the effectiveness of seed inoculation with *Azotobacter* and other bacteria is now being ascribed, in part at least, to the control of pathogenic fungi, perhaps by antibiosis (Mishustin and Naumova, 1962).

Inoculation of seed with organisms of known antibiotic potential has been successful in the control of certain diseases, for example fusarium blight of oat seedlings (Tveit and Wood, 1955), which has been controlled as effectively by inoculation of the seed with isolates of *Chaetomium*, as by mercurial seed dressings. The lack of clear correlation between in vitro and in vivo activity of different *Chaetomium* isolates may have been due, as suggested by Tveit and Wood, to the binding of antibiotic to the hyphae, or perhaps to a failure to include root exudates in the test media.

In exploring the possibilities of disease control by introducing antagonists into the rhizosphere, the potentialities of strains of fungi known to be normal colonizers of the root surface have not yet received sufficient attention. As an example, *Fusarium oxysporum* and *Cylindrocarpon radicicola* are two of the most abundant rhizoplane inhabitants of grasses and cereals as well as other crops in many soils. Strains of both of these species show antifungal activity (Buxton, 1960; White, Chilvers, and Evans, 1962), which may contribute to their status as successful root colonizers. Selection of active antifungal strains of either of these organisms, followed by seed inoculation, could form a useful basis for work on biological control. Ectotrophic mycorrhizal fungi may constitute a defensive barrier against pathogenic fungi, for there is evidence both of the production of antibiotics by these fungi (Santoro and Casida, 1959) and of a marked modification of the microflora in the vicinity of mycorrhizal roots (Robertson, 1954). Inoculation with specific mycorrhizal fungi could perhaps be a factor in decreasing disease susceptibility.

Alteration of the normal fungistatic equilibrium in soil offers a possible approach to the control of some soil pathogens. Chinn and his colleagues (1953) found that helminthosporium root rot of wheat seedlings could be controlled by adding to the soil organic amendments such as soybean meal. The effect of these additions was to induce germination in fungistatically inhibited conidia of the root-rot fungus. After germination, lysis of germ tubes occurred, resulting in a reduction in the population of the fungus. In later studies Chinn and Ledingham (1957, 1961) found that the most effective substances in promoting germination of *Helminthosporium sativum* conidia in soil were natural products, such as wheat germ, bran, molasses. They suggested that some of the effects of green manuring might be explained by stimulation of germination, which is then followed by lysis. Various crop residues can stimulate, to differing degrees, the germination of inactive chlamydospores of *Fusarium solani* f. *phaseoli* (Toussoun, Patrick, and Snyder, 1963), the germ tubes formed sometimes then undergoing lysis. The situation is, however, complicated by the fact that substances from decomposing plant residues may increase the susceptibility of host tissues to invasion by fungal pathogens (Toussoun and Patrick, 1963).

Since the proximity of roots may also provide a stimulus to the germination of inhibited spores or other resting bodies in soil (Jackson, 1960; Schroth and Snyder, 1961; Shreiber and Green, 1963), trap-cropping with stimulatory but nonhost species to reduce soil infestations of a pathogenic fungus offers another possible avenue of control. Success depends upon three conditions: stimulation of germination in the rhizosphere of the "bait" plant, noninfection of this plant, and inability to reproduce in its rhizosphere. Unfortunately, there is evidence, as Schroth and Snyder have shown, that even when the first two conditions are satisfied, saprophytic growth and reproduction can lead to an actual increase in the infestation.

A consideration of all the evidence we now have must convince us that antibiosis, in one form or another, plays a key role in the ecology of soil microorganisms, being perhaps second in importance only to

competition for nutrients. Increasing knowledge of the interrelations and dynamics of the rhizosphere population will lead to increasing possibilities for the biological control of root diseases through antibiosis.

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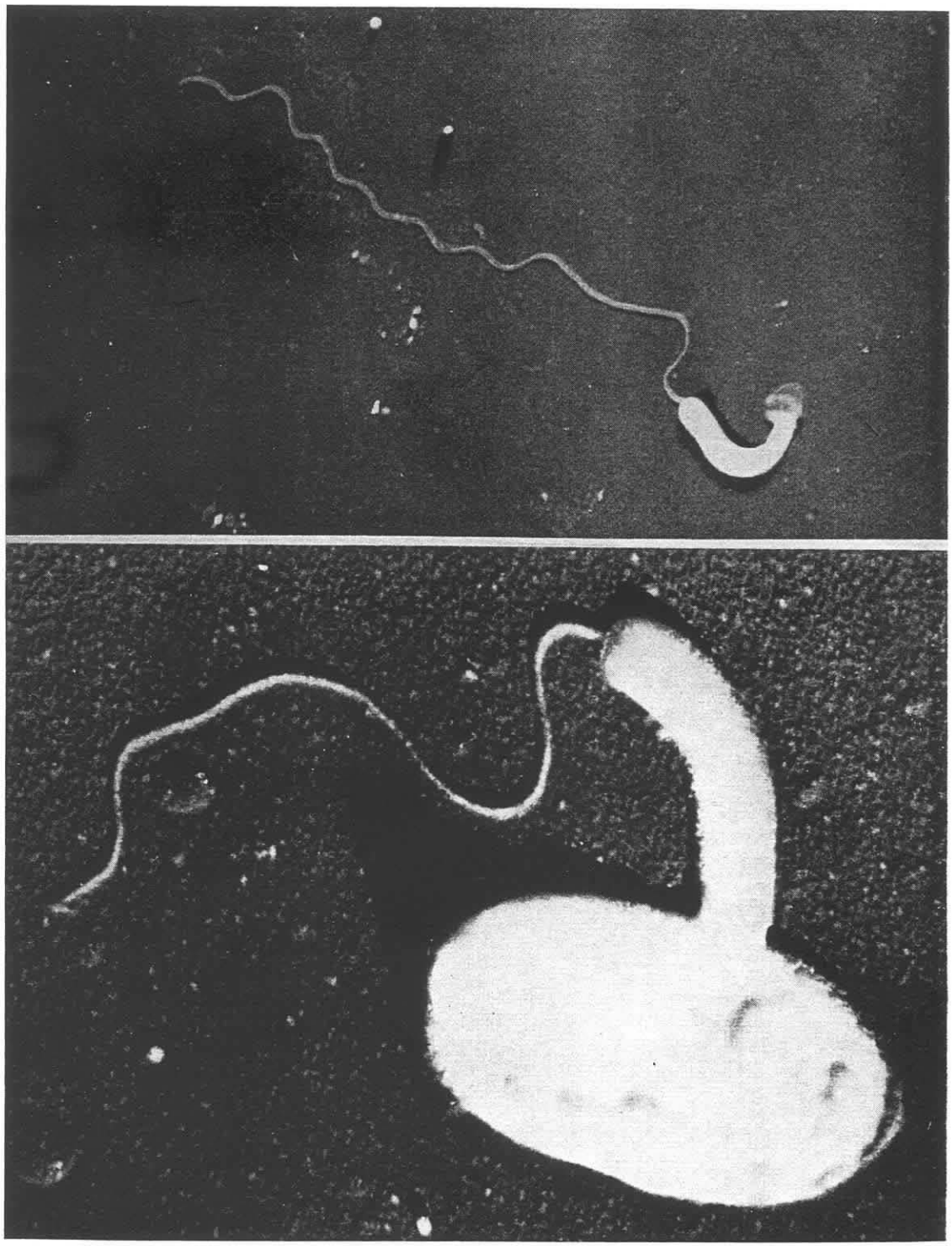
► DISCUSSION OF R. M. JACKSON PAPER

H. Stolp:

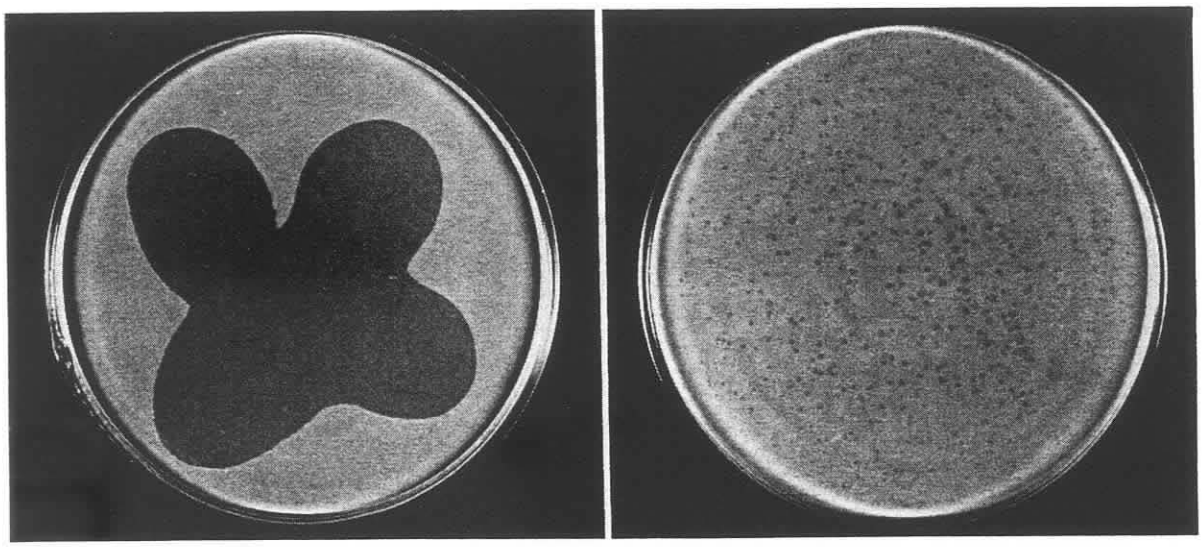
A special type of antagonistic relation among bacteria is represented by the parasitic action of a vibrio-like organism (Fig. 1) on susceptible host bacteria. The parasite is of minute size (0.3 × 0.8 μ approximately), and possesses a flagellum of about 50 mμ in diameter. It is highly motile and attaches with the nonflagellated end to the bacterial cell surface (Fig. 2), inducing in Gram-negative bacteria spheroplast formation and finally complete lysis (Fig. 3). In a bacterial lawn, the plating of a dilution of the parasite results (with appropriate dilutions) in the formation of "plaques" (Fig. 4) that externally are not distinguishable from the known phage-plaques. In liquid culture, the action of the parasite is accompanied by a reduction in optical density. The first strain, isolated in Berlin, shows activity restricted to bacteria belonging to the pseudomonads. Since then, in collaboration with M. P. Starr at the University of California, Davis, a number of strains of this parasite have been isolated that attack bacteria of different systematic position and that differ in their host-activity spectra (H. Stolp and M. P. Starr. Antonie van Leeuwenhoek J. Microbiol. Serol. 29: 217-248. 1963.). The possibility was indicated that this type of antagonism may have some ecological importance with respect to changes of bacterial equilibria.

S. Ishizawa:

I should like to comment on the distribution in soil of actinomycetes producing antibiotic substances against fungi or bacteria. Although the number of microorganisms decreases with depth, there exists some evidence



Figs. 1 and 2. Electron micrographs of *Bdellovibrio bacteriovorus* strain 100. (H. Stolp and M. P. Starr. Antonie van Leeuwenhoek J. Microbiol. Serol. 29: 217-248. 1963.) **Fig. 1** (upper). Single individual (× 18,000). **Fig. 2** (lower). *Bdellovibrio* attached to a host cell of *Erwinia amylovora* (× 33,000).



Figs. 3 and 4. Lytic effect of *Bdellovibrio bacteriovorus* on bacteria in culture. (H. Stolp and M. P. Starr. Antonie van Leeuwenhoek J. Microbiol. Serol. 29: 217-248. 1963.) **Fig. 3** (left). Lytic areas in a lawn of *Escherichia coli* B, produced by *B. bacteriovorus* strain Bd. 109. A suspension containing the parasites was dropped onto the bacterial lawn. **Fig. 4** (right). Single plaque formation by *Bdellovibrio* strain A3.12 on its homologous host, strain A3.12.

that the actinomycete flora differs considerably among horizons. For instance, according to one of the results obtained in our laboratory, the proportion of actinomycetes which inhibit strongly the growth of *Erwinia* is evidently larger in the lower horizon.

For biological control of plant pathogens in soil, study of the microbiological characterization of soil on the basis of the composition of soil population as well as on its activity should be intensified. It will be enhanced by combining microbiological criteria with chemical or physical criteria in the pedological classification of soil.

J. L. Lockwood:

I should like to discuss some of our results which may bear on the source of soil fungistasis. Natural soil amended with washed or unwashed conidia of any of several fungi gave increased oxygen uptake in Warburg flasks, as compared with nonamended soil. A portion of this increased respiration may be due to respiration of spores themselves, but stimulation of oxygen uptake can also be achieved by amending soil with sterile, aqueous washings from fungus spores. Similar results were obtained with autoclaved spores and dilute peptone solution.

Increased numbers of bacterial colonies were obtained in soil dilution plates after amendment of natural soil with spores and incubating for 8-16 hours.

Inhibition of germination of fungus conidia was obtained when a mixed population of bacterial cells was washed and centrifuged several times with dilute buffer, then incubated with the spores. But when the washed bacterial cells were incubated for 6 hours, then sterile-filtered, and conidia of *Helminthosporium* (which germinated in 2 hours) were placed in the filtrate, germination was not inhibited.

Germination of washed conidia on natural soil surfaces sometimes exceeded that of nonwashed conidia, and spores capable of rapid germination (e.g. rust uredospores) sometimes gave up to 50% germination on natural soil surfaces.

In some preparations of spores removed from natural soil surfaces on thin films, areas of bacterial proliferation could be seen.

These results are consistent with the concept that spores provide food for soil microbes, which, in turn, inhibit the spores in some manner.

C. G. Dobbs:

I prefer the word mycostasis to fungistasis. The phenomenon intervenes when and where other conditions for the initiation of growth are met, and it generally has a "competitive" relation with certain nutrients, notably sugars. It is important, however, to realize that many soils contain a residual mycostasis, not heat-sensitive or broken by sugars, and probably mainly inorganic in origin. This may well also exist in agricultural or treated soils and should always be investigated. It is now clear that many microorganisms may be involved in the production of the general, heat- and sugar-sensitive soil mycostases. Lockwood's evidence that freshly added spores may stimulate microbial soil extracts which show an increased inhibition when the soil is "fed" with washed spores of the test fungus, does not explain the continued inhibition of old spores in the soil. The concept of staling of the microhabitat cannot be excluded by work on fresh, cultured spores.

R. R. Baker:

It is essential in studies of soil fungistasis to indicate whether washed or unwashed material is used for testing, since nutrient may be carried over on the spores. Fungi found in soil should be used since these often have characteristics different from those not ordinarily found in this habitat. G. J. Griffin, in unpublished work, has further evidence for fungistasis being similar to staling. Sterile soil extracts, obtained with a pressure-membrane apparatus, contain inhibitory factors. Use of a sausage-casing membrane contributes nutrients tending to mask this, however. It is difficult to conceive of leakage of nutrients from prop-

agules in soils promoting bacterial growth for long periods of time.

G. D. Pentland:

Dr. Kerr has noted the effect of discontinuous soil water on the availability of food for microorganisms, and on limiting bacteria to water pockets. Previously, Dr. Dobbs has gone on record as indicating that the fungistatic substance, or substances, in soil must be water-soluble. Would you comment on the effect of limited soil water on the availability of antibiotics or fungistatic substances—not so much in terms of production as in terms of diffusion through soil and contact with hyphal strands, especially when the water becomes discontinuous? We have been doing some experiments on the mycelial spread of *Coniophora puteana* from a wood food base through soil at different moisture levels. It will spread through the drier treatments but not through the wetter ones. We have visualized the reason for this as being connected with a discontinuity of the fungistatic substances in the drier soil.

R. M. Jackson:

Yes, it would seem likely that most antibiotics, or fungistatic substances, would depend on continuous water films for diffusion through soil. Under dry conditions, such diffusion would undoubtedly be impeded or prevented.

C. W. Emmons:

The fungi which cause systemic mycoses are saprophytes in soil or organic debris and these environmental sites, rather than infected man or animals, are the sources of infection for man and animals. In certain habitats these potential pathogens are very numerous. *Aspergillus fumigatus* may represent 95% of the viable spores at one stage in the succession of fungi in a compost pile. We have found 50,000,000 viable cells of *Cryptococcus neoformans* per g of an old pigeon nest. Here (at least ephemerally) competition seems to be absent or unimportant. On the other hand, *Histoplasma capsulatum* grows also in restricted habitats, but in the presence of many other fungi. We have been studying the occurrence of *H. capsulatum* around a house where human cases of histoplasmosis occurred, and in a small park adjacent to Pennsylvania Ave., Washington, D.C. The presence of house bats and roosting starlings, respectively, are the ecologic factors which enrich these soils and permit *H. capsulatum* to compete with other saprophytes. We should like to find a method of biological control which would eradicate or reduce the population of this pathogen without using general poisons which kill shrubbery and trees. We have isolated several species of *Streptomyces* from soil containing *Histoplasma*. These inhibit *Histoplasma* when grown in vitro with *Histoplasma*, but we have not been able to isolate an active antibiotic nor to demonstrate a change in the microflora of the soils supporting *Histoplasma* after heavy seeding, with and without organic amendments, with these strains of *Streptomyces*. We recognize that such a failure is a common experience.

J. Ulrich:

I should like to report on the activity of enzymes at the surfaces of colloids, as done in Dr. A. D. McLaren's laboratory. It was found that enzymatic activity was maintained even while the enzymes were adsorbed. If antibiotics were likewise adsorbed to soil colloids, they could possibly act from the adsorbed position and not necessarily have to be released to exert antibiotic activity on other organisms. Possibly the colloids would exert a "protective" action on the antibiotics.

J. Altman:

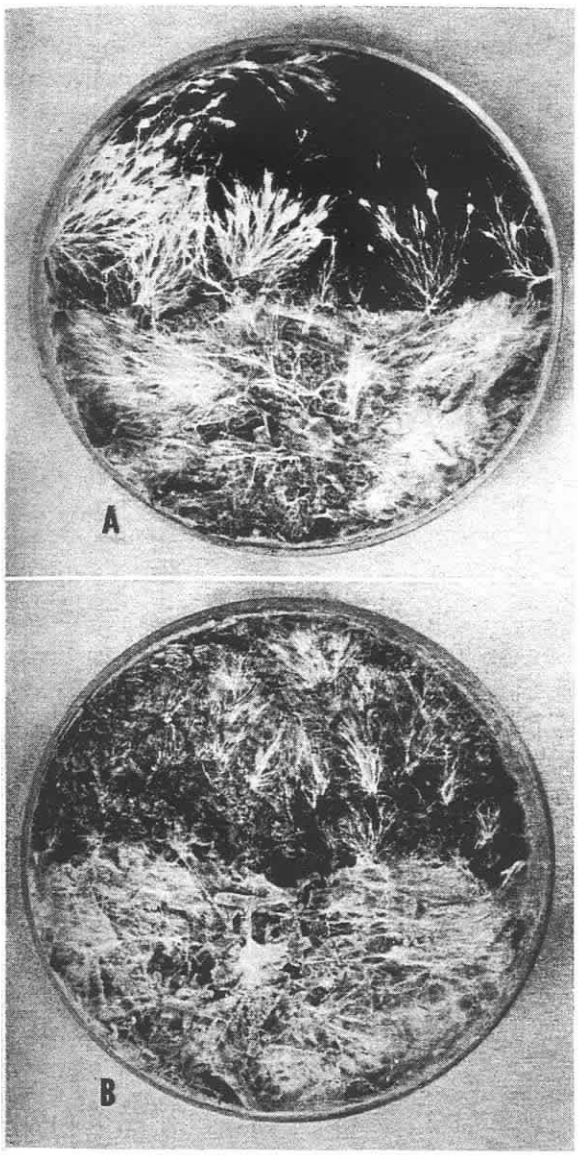
All discussion regarding fungistasis (mycostasis) seems to imply raw or nonsterile soil as being prerequisite, and from all pertinent discussion following your paper, a labile factor, also. Since this phenomenon is widespread and occurs under varying conditions, is reversed by nutrients, and affected by oxygen, is it not conceivable that every living microbe in soil is capable of releasing CO_2 in its metabolism? This CO_2 can readily be converted to H_2CO_3 in the water film or phase in soil. Secondly, some organisms might give off ethylene gas; both CO_2 and possibly ethylene can arise as a part of the glycolytic cycle. Sugar could overcome this fungistasis by stimulating growth of saprophytic microorganisms which could readily utilize CO_2 or break down ethylene. ???

Gerlind Eger: (A color motion picture was shown.)

Agaricus bisporus grows on composted or sterilized organic materials. As a rule, fructification occurs if the mycelium is covered with a layer of suitable unsterile casing material, for example, garden soil. By use of the *Halbschalentest* it is possible to obtain on a laboratory scale a quasi cross-section through the upper 6 inches of a mushroom bed. The mycelium is cultivated on sterilized compost in one half of a petri dish, the other half is filled with moist casing soil. After casing, the mycelium grows from the compost to the soil. After a few days of growth with normal speed, the growth becomes slower in certain areas as if it is being inhibited. In some instances, growth of the entire advancing front of mycelium slows down. Where the growth is inhibited, the mycelium forms minute white nodules, the fruitbody initials. The soil ahead of the initials mostly remains black because of the absence of mycelium growing on it. (Fig. 5, *A*.)

In some experiments, casing soil from the black areas of petri dishes, near where fruitbody initials were forming, was suspended in water. One part of the suspension was poured on heat-sterilized soil; this served to initiate fruitbodies in subsequent tests. The second part was filtered through paper filters and then poured on sterile soil, fruitbody formation also was initiated. The third part was sterilized by filtration through Seitz EK filters; no fruitbody initials were formed (Fig. 5, *B*). It is concluded that living microorganisms cause the initiation of fructification. On the other hand, growing mycelium of the cultivated mushroom inhibits the growth of microorganisms by means of a volatile substance. Evidence was given by the following experiments: Each of two series of agar plates in petri dishes was inoculated with 1 ml of the suspension of a microorganism. In one series growing mycelium of *Agaricus bisporus* was put on the lid. In the dishes containing growing mycelium, growth of the tested bacteria and molds was inhibited.

These and other experiments suggest that a specific microflora in the casing layer is selected by the volatile substance emitted by the mushroom mycelium when it grows under optimum conditions. If, however, the aeration is insufficient, the volatile substance accumu-



lates and even the growth of the favorable organisms is inhibited.

To obtain fructification of *Agaricus bisporus,* a balance must be maintained between the mushroom mycelium and the microflora in the casing layer.

L. C. Schisler:

I agree with Dr. Eger's findings of antibiotic activity of the mycelium of the cultivated mushroom, *Agaricus campestris* var. *bisporus.* This is readily observable and can be demonstrated in a variety of ways.

However, concerning her postulation that the microflora of the soil are responsible for the fruiting initiation in the cultivated mushroom, I have two questions:

1. What organisms are involved and can this effect be reproduced in pure culture?

2. How does she explain the initiation of fruiting under aseptic conditions observed by several investigators in this field?

G. Eger:

The organisms in question are bacteria or actinomycetes or both, but it has not yet been possible to obtain pure cultures.

There is no evidence, in some experiments with aerated cultures, that the conditions remained sterile until the end of the experiments. It is to be expected that the effect of the microorganisms in the casing layer of mushroom cultures can be replaced by chemical treatment under aseptic conditions. In my own experiments (Arch. Mikrobiol. 39: 313-334, 1961; Naturwissenschaft 49: 261, 1962) poor fruiting was obtained under sterile conditions with moist charcoal. Dr. E. Hauser (Switzerland) told me in 1959 that she had a mushroom strain that formed sporophores on sterile grain spawn in bottles. This is a great exception. Normal mushroom strains never do this. A single sporophore, even on horse-manure spawn, very scarcely occurs and was always reported to be a great exception. But in all these cases an incidental contamination was not excluded.

Fig. 5. The *Halbschalentest* in which the mushroom grows on compost confined to the lower half of the dish. When casing soil is added to the upper half of the dish, mycelium grows over it: *A,* with nonsterile casing soil the strands form fruitbody initials in response to inhibition of growth. *B,* if the casing soil is sterile throughout fruitbody initials do not develop.